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REMARKS

A complete listing of the all the claims in this application is provided above. In the complete listing, all previous claims have been canceled. The canceled claims above include the previously allowed claims 576, 578-596, 598-617, 639-658, 660-677, 679-697, 699-716, 718-736, 738-755, 757-775, 777-794 and 796-825.¹ The new claims presented are numbered 826-1227.

New Claims

New claims 826-1227 are drawn to corresponding subject matter in the previous and now canceled claims. Among the new claims are independent claims 826, 856, 888, 921, 956, 988, 1022, 1054, 1088, 1121, 1156 and 1191 that correspond to the previous and now canceled claims 576, 596, 617, 637, 658, 677, 697, 716, 736, 755, 775 and 794, respectively. For the Examiner's convenience, a table has been prepared to show the correspondence between each of the new claims and the previous claims. The table is attached as Exhibit 1. Any changes in the language of corresponding claims are listed in the third column of the table.

Three changes in the independent claims merit mention at the outset. **First**, the meaning of the term "analog" has been clarified both with respect to the nucleotide and to the base moiety (or "nucleobase" as it is termed in the Office Action, page 3, second paragraph)². This matter is discussed further below in the indefiniteness rejection (page 86, fourth full paragraph, through page 87, last full paragraph, through page 90, line 2). To clarify the language, Applicants have

¹ The allowance of these claims was withdrawn by the Director of Group 1600 in a notice dated May 16, 2003.

² This change arises out of a similar issue in related U.S. Patent Application Serial No. 08/486,069 (filed June 7, 1995) belonging to the same family and claiming priority of U.S. Patent Application Serial No. 06/391,440, filed on June 23, 1982.

omitted the phrase "or analog thereof" from each of the independent claims (826, 856, 888, 921, 956, 988, 1022, 1054, 1088, 1121, 1156 and 1191).

Furthermore, the term "base analog" is now recited in the independent claims, together with specific base analogs that include a pyrimidine analog, a purine analog and a deazapurine analog. Thus, each of the independent claims (826, 856, 888, 921, 956, 988, 1022, 1054, 1088, 1121, 1156 and 1191) now recite that

. . . BASE is a base moiety or a base analog comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA . . .

Support for the foregoing language is found throughout the specification. Listed below are numerous instances where nucleotide analogs and particularly, base analogs (nucleobase analogs), are described in the specification.

Specification References to Nucleotide Analogs And/Or Base Analogs

<u>Description</u>	<u>Page/Para./Line</u>
analogs of dUTP and UTP (emphasis added)	Page 1, 10th line from bottom
the analogs must be relatively efficient substrates	Page 7, line 9
5-methylcytosine, and 5-hydroxymethylcytosine (emphasis added)	Page 9, 2nd & 3rd lines from bottom
thymidine analog (emphasis added)	Page 31, line 14
analogs of dUTP and UTP (emphasis added)	Page 37, 12th line from bottom

<u>Description</u>	<u>Page/Para./Line</u>
5-hydroxy-methylcytosine (5 HMC)	Page 54, 2nd and 3rd full paragraphs
reacting nucleic acids in the double helical form with alkylating agents as for example benz(o)pyrene diol epoxide or aflatoxin. Under appropriate conditions the N ² group of guanine, the N ⁴ group of adenosine or the N ⁴ group of cytosine are alkylated	Page 54, last paragraph
5-Hydroxymethyl-2'-deoxycytidylic acid	Page 60, Example X
5-(4-aminobutylaminomethyl)-2'-deoxyuridylic acid	Page 61, Example XI
Biotinylated-5-(4-aminobutylaminomethyl)-2'-deoxy- uridylic acid	Page 61, Example XII
5-formyl-2'-deoxyuridine	Page 62, Example XIII
Biotinylated 5-formyl-2'-deoxyuridine	Page 63, Example XIV
Biotinylated 5-amino-2'-deoxyuridine	Page 63, Example XV
5-(oxy)acetic acid-2'-deoxyuridine	Page 64, Example XVI
Biotinylated 5-(oxy)acetic acid-2'-deoxyuridine	Page 64, Example XVII
5-hydroxymethyl-2'-deoxycytidine-5'-triphosphate	Page 66, Example XIX
maltotriose nucleotide [maltotriose coupled 5-(3-amino- 1-propenyl)-2'-deoxyuridine-5' triphosphate	Page 71, Example XXIII
5-(perfluorobutyl)-2'-deoxyuridine	Page 72, Example XXIV
Tubericydin (emphasis added)	Page 72, Example XXV
Toyocamycin (emphasis added)	Page 72, Example XXV
Maltotriose coupled 5-(3-amino-1-propenyl)-2'-deoxy- uridine-5' triphosphate	Page 75, Example XXXI
Fluorescein coupled 5-(3-amino-1-propyl)-2'-deoxy- uridine-5'-triphosphate (AA-dUTP)	Page 76, Example XXXII

<u>Description</u>	<u>Page/Para./Line</u>
5-Bromo-2'-deoxyuridine-5'-phosphate (emphasis added)	Page 78, Example XXXV
6-Cyano-2'-deoxyuridine-5'-phosphate	Page 79, Example XXXVII
6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid	Page 80, Example XXXVIII
Two minor purines	Page 91
2-Methyladenine	
1-Methylguanine	
Two minor pyrimidines	Page 91
5-Methylcytosine	
5-Hydroxymethylcytosine	
A nucleotide . . . wherein said base B is 2-methyladenine	Original Claim 71
A nucleotide . . . wherein said base B is 1-methylguanine	Original Claim 71
A nucleotide . . . wherein said base B is 5-methylcytosine	Original Claim 72
A nucleotide . . . wherein said base B is 5-hydroxymethylcytosine	Original Claim 73

Applicants would respectfully point out that several of the above-listed and boldfaced nucleotide analogs or base analogs (nucleobase analogs) are similar if not identical to those found in Dr. Arthur Kornberg's DNA Replication published in 1980 [W. H. Freeman And Company, San Francisco, Chapter 12, "Inhibitors of Replication," pages 415-441]. On page 424 in Figure 12-4 which is titled "Nucleotide analogs incorporated into DNA or RNA," Dr. Kornberg provides numerous examples of base analogs of uridine and thymidine, base analogs of adenine and base analogs of adenosine, and these include the 14 or so base analogs listed below:

Enz-5(D6)(C2)

Base analogs of uridine & thymidine

5-Hydroxy-, 5-Amino-, 5-Bromo-, 5-Iodouracil

5-Hydroxy-, 5-Amino-, 5-Bromo-, 5-Iodothymidine

Base analogs of adenine

2-Aminopurine

2-Aminoadenine

Base analogs of adenosine

Tuberacidin

Formycin

Toyocamycin

7-Deazanebularin

A copy of pages 423-427, including the aforementioned Figure 12-4 from Dr. Kornberg's DNA Replication book, is attached to this paper as Exhibit 2.

Nucleotide analogs and base analogs are also defined in the new claims as those analogs which "can be attached to or coupled to or incorporated into DNA or RNA. Furthermore, such analogs are also defined as not substantially interfering "with double helix formation or nucleic acid hybridization." An artisan working in the field of nucleic acids technology, including nucleic acid modifications and labeling for use in hybridization detection, sequencing and other processes, would appreciate and understand the meaning and extent of the foregoing claim language. Moreover, such an artisan working in the field of nucleic acids would also appreciate and understand from this claim language which members would be included in such nucleotide analogs and which members would be excluded.

With respect to the attachment, coupling or incorporation of nucleotide analogs and base analogs as recited in the new claims, the specification is replete with support for this language. In fact, the specification describes numerous instances for the attachment, coupling or incorporation of modified nucleotides or nucleotide analogs into DNA or RNA. These instances are set forth below.

Specification References to Attachment, Coupling and Incorporation

<u>Description</u>	<u>Page/Line</u>
nucleotides are modified, such as at the 5 position of pyrimidine or the 7 position of purine, preparatory for the preparation for the preparation therefrom of nucleotide probes suitable for attachment to or incorporation into DNA or other nucleic acid material	Page 52, last paragraph (under "Summary of the Invention")
Biotin and polybiotinylated poly-L-lysine were coupled to oligoribonucleotides	Page 57, Example V
Formaldehyde coupling of cytochrome C-biotin and polybiotinylated poly-L-lysine to oligodeoxyribonucleotides were carried out	Page 58, Example VII
Ligation of poly dA:poly dT, biotinyl dU to oligo-deoxyribonucleotides was accomplished	Page 60, Example IX
labeling purified DNA [by nick translating] with biotinylated 5-formyl-2'-deoxyuridine	Page 67, Example XX

<u>Description</u>	<u>Page/Line</u>
Lambda DNA was nick translated as described herein with maltotriose coupled to 5-(3-amino-1-propenyl)-2'-deoxyuridine-5' triphosphate and 3H-2'-deoxyadenosine	Page 71, Example XXIII
A DNA probe was ligated to a synthetic DNA composed of repeated sequences of <i>E. coli</i> lac operator DNA	Page 77, Example XXXIV
As indicated hereinabove, various techniques may be employed in the practices of this invention for the incorporation of the special nucleotides of this invention into DNA and related structures. One particularly technique referred to herein involves the utilization of terminal transferase for the addition of biotinated dUMP onto the 3' ends of a polypyrimidine or to single-stranded DNA. The resulting product, such as a single-stranded or cloned DNA, which has biotinated dUMP onto the 3' ends thereof, can be recovered . . .	Page 99, second paragraph
These nucleotides are then incorporated into specific nucleic acids using a DNA or RNA polymerase or ligase reaction or a chemical linkage.	Page 101, first paragraph (third sentence)

<u>Description</u>	<u>Page/Line</u>
A nucleotide in accordance with Claim 1 wherein . . . such that when said nucleotide is incorporated into or attached to or associated with a double-stranded deoxyribonucleic acid or double-stranded ribonucleic acid or DNA-RNA hybrid, . . .	Original claim 7
A ribonucleotide in accordance with Claim 143 wherein when said nucleotide is incorporated into or attached to a double-stranded deoxyribonucleic acid or double-stranded ribonucleic acid or DNA- RNA hybrid, . . .	Original claim 145

The literature, which includes an entire textbook devoted specifically to nucleotide analogs (and actually titled in part Nucleotide Analogs³) as well as other well-known textbooks by the Nobel Prize winning author and scientist, Dr. Arthur Kornberg of Stanford University, is also replete with references to nucleic analogs and more particularly, nucleotide analogs which can be attached to or coupled to or incorporated into DNA or RNA or other nucleic acid or genetic structures or material. See Dr. Kornberg's Figure 12-4 on page 424 of his book cited above and provided in Exhibit 2. The title of this Figure is "Nucleotide analogs incorporated into DNA or RNA."

³ The full name for this textbook published before the June 23, 1982 priority filing date of the instant application is Nucleotide Analogs: Synthesis and Biological Function [Dr. Karl Heinz Scheit, John Wiley & Sons, Inc., New York, 1980, 288 pages]. Dr. Karl Heinz Scheit was Professor at the Max-Planck-Institut fur Biophysikalische Chemie in Gottingen, Germany, and he provides a comprehensive account of nucleotide analogs that covers almost 300 pages.

Second, in the new claims above, the term "sugar moiety" has been changed to -- furanosyl moiety --.⁴ It is believed that the new term -- furanosyl moiety -- more accurately describes the nucleic acid sugars which enable the skilled person in the art to make/use the present invention commensurate in scope with the new claims. Moreover, the term "furanosyl moiety" is literally supported by the structures in the specification. See the funanosyl structures depicted in the specification, pages 6 and 38. See also originally filed claim 46. The term "furanosyl moiety" is accepted in the art to describe the sugars present in nucleic acids. See, for example, Wolfram Saenger's description in his book, Principles of Nucleic Acid Structure [Springer-Verlag, New York, 1984, page 1]:

A nucleotide consists of three molecular fragments: **sugar**, heterocycle, and phosphate. The **sugar, ribose or deoxyribose**, is in a **cyclic, furanoside form** and is connected by a β -glycosyl linkage with one of four heterocyclic bases to produce the four normal nucleosides: adenosine, guanosine, cytidine, and thymidine (uridine in ribonucleic acid, RNA). If the 3' or 5'-hydroxyl group of **sugar** is phosphorylated, we have a nucleotide. . . [emphasis added; copy attached as Exhibit 3]

See also the definition of "furanose" in Stenesh's Dictionary Of Biochemistry And Molecular Biology [John Wiley & Sons, New York, 1989, page 188; copy attached as Exhibit 4]:

furanose A monosaccharide having a five-membered ring structure.

Third, the non-radioactive label moiety Sig has been further clarified in the new claims. Thus, Sig is now defined as a "non-polypeptide, *non-nucleotidyl*, non-radioactive label moiety which can be directly or indirectly detected . . ."

Applicants respectfully point out that the term "non-nucleotidyl" is only further defining the nature of the non-radioactive signal, more particularly, the non-

⁴ A similar enablement rejection was made in related U.S. Patent Application Serial No. 08/486,069, cited *infra*.

radioactive label Sig moiety. The inserted term -- non-nucleotidyl -- is supported by the specification. For example, members of the non-radioactive label moiety Sig are described on several pages in the specification. For example, on page 10, it is disclosed:

A may be any moiety which has at least three carbon atoms and is capable of forming a detectable complex with a polypeptide when the modified nucleotide is incorporated into a double-stranded duplex containing either deoxyribonucleic or ribonucleic acid. . . .

Of these preferred A moieties are **biotin** and **iminobiotin**.

[emphasis added]⁵

See pages 96, last paragraph, continuing through page 97, first paragraph, and each of these members are non-nucleotidyl in nature:

The Sig moiety employed in the make-up of the special nucleotides of this invention could comprise an enzyme or enzymic material, such as alkaline phosphatase, glucose oxidase, horseradish peroxidase or ribonuclease. The Sig moiety could also contain a **fluorescing component**, such as fluorescein or rhodamine or dansyl. If desired, the Sig moiety could include a **magnetic component** associated or attached thereto, such as a magnetic oxide or magnetic iron oxide, which would make the nucleotide or polynucleotide containing such a magnetic-containing Sig moiety detectable by magnetic means. The Sig moiety might also include an **electron dense component**, such as ferritin, so as to be available by observation. The Sig moiety could also include a radiation detecting means. The Sig moiety might also include a hapten component or per se be capable of complexing with an antibody specific thereto. Most usefully, the Sig moiety is a

⁵ Biotin and iminobiotin are listed among the members for Sig in several new dependent claims. See, for example, new claim 828.

polysaccharide or oligosaccharide or monosaccharide, which is capable of complexing with or being attached to a sugar or polysaccharide binding protein, such as a lectin, e.g. Concanavilin A. The Sig component or moiety of the special nucleotides in accordance with this invention could also include a **chemiluminescent component**.

[emphasis added]⁶

Entry of the new claims is respectfully requested.

Drawings Objection

Acknowledgement is made of the sheet (Attachment for PTO-948 (Rev. 03/01 or earlier) that was attached to the November 26, 2003 Office Action. In response to the drawings objection, Applicants submitted a new set of drawings on December 4, 2003. The paper was titled "Transmittal of Formal Drawings" and it included new Figures 1-4.

The Rejection Under 35 U.S.C. 112, Second Paragraph

Claims 576, 578-596, 598-617, 619-637, 639-658, 660-677, 679-697, 699-716, 718-736, 738-755, 757-775, 777-794, and 796-825 stand rejected under 35 U.S.C. §112, second paragraph, for indefiniteness. In the Office Action (pages 2-3), the Examiner stated:

Claim 595 depends from claim 576 which is comprised of an oligo- or deoxyribonucleotide further comprising at least one Sig modified nucleotide. Claim 595 is vague and indefinite as to what is meant by requiring that said oligo- or polynucleotide comprises at least one ribonucleotide. Since the only two components cited in claim 576 are either an oligo- or polynucleotide and one or more Sig modified

⁶ The boldfaced elements are also recited as members of Sig in several new dependent claims. See, for example, new claim 828.

nucleotide, it is unclear whether the ribonucleotide cited in claim 595 is a further comprising component of the claimed polymer or whether this is meant to limit the one or more Sig modified nucleotide(s). Thus the antecedent basis for the ribonucleotide limitation in claim 595 is unclear. It is noted that claim 595 does not cite the at least one ribonucleotide as "further comprising" regarding the claimed polymer content. Clarification via clearer claim wording is requested. This unclarity is also present equivalently in instant claims 615, 676, 695, 754, and 773.

All of the independent claims cite the phrase "analog thereof" regarding BASE moieties therein. Consideration of the instant disclosure as filed as failed to reveal any definition of the metes and bounds of what is meant thereby. What constitutes an analog of a nucleobase vs. a moiety which is not an analog. Do analogs participate in hydrogen bonding or not? Is steric hindrance a defining feature regarding whether hybridization must be available for such analogs in duplex formation or not? Clarification via clearer claim wording is requested. This issue also applies to claims which depend directly or indirectly from the independent claims due to their dependence.

The indefiniteness rejection is respectfully traversed.

As set forth in the new claims above and in the attached table (Exhibit 1), claims 855, 886, 918, 1018, 1120 and 1154 correspond to the former rejected claims 595, 615, 676, 695, 754, and 773, respectively.

In response to the first part of the indefiniteness rejection, Applicants have inserted the word "further" before "comprising," as suggested by the Examiner.

This change is reflected in each of new claims 855, 886, 918, 1018, 1120 and 1154.

Regarding the second part of the indefiniteness rejection and the phrase "analogs thereof" as it previously defined "BASE moiety," Applicants have effected a number of changes reflected in the new claims as set forth above and in the attached table (Exhibit 1). **First**, their claimed compositions all recite "at least one modified nucleotide or modified nucleotide analog," in keeping with the support in the specification. See discussion above, pages 80-82.

Second, BASE is defined as a "base moiety or a base analog comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization." Again, the foregoing recitation for BASE as a base moiety or a base analog is well supported by the specification and the several examples of BASE analogs that are disclosed therein. See above discussion on pages 80-82.

Third, several dependent claims added to the new claims provide embodiments for BASE analogs. For example, new claims 850-854 are directed to specific pyrimidine analogs (thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs and deoxycytidine analogs); specific uridine analogs (5-bromo-2'-deoxyuridine-5'-phosphate); specific deoxycytidine analogs (5-hydroxymethyl-2'-deoxycytidylic acid); specific purine analogs (adenosine analogs, deoxyadenosine analogs, guanosine analogs and deoxyguanosine analogs); and specific adenosine analogs (tubercidin and toyocamycin).

See other dependent claims 881-885, 912-916, 946-950, 979-983, 1012-1016, 1045-1049, 1078-1082, 1115-1119, 1149-1153, 1180-1184 and 1216-1220.

In light of the presentation of the new claims, Applicants respectfully request reconsideration and withdrawal of the indefiniteness rejection.

The Rejection Under 35 U.S.C. 112, First Paragraph

Claims 576,578-595,617,619-636,658,660-676,697,699-715, 736, 738-754, 775, and 777-793 stand rejected on enablement grounds under 35 U.S.C. §112, first paragraph. In the Office Action (page 3-4), the Examiner stated:

. . . the specification, while being enabling for nucleotide containing embodiments wherein the sugar is a furanose moiety, such as ribose or deoxyribose, does not reasonably provide enablement for any generic sugar, such as cited in claims 576 etc. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make/use the invention commensurate in scope with these claims.

On pages 4-5, the Examiner went to state further:

Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized in Ex parte Forman, 230 USPQ 546 (BPAI1986) and reiterated by the Court of Appeals in In re Wands, 8 USPQ2d 1400 at 1404 (CAFC 1988). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

The Board also stated that although the level of skill in molecular biology is high, the results of experiments in genetic

engineering are unpredictable. While all of these factors are considered, a sufficient amount for a prima facie case are discussed below.

Upon reconsideration of the instantly claimed sugar (SM) structures revealed that only furanose sugar moieties are disclosed which form the SM structure in oligonucleotides or polynucleotides for which attachment and/or hybridization ability are reasonably enabled. It is also noted that the early biochemical textbook of Lehninger summarized the structure of nucleic acids such, as DNA on pages 638-639 as a specific structure. It is noteworthy that even though this textbook is over 30 years old the only notable backbone structure analog to be significantly utilized in biochemical reactions in that of peptide nucleic acids. These peptide nucleic acids, however, lack any sugar in the backbone, but rather utilize peptide bonds with spacing linkages and thus is not a nucleotide polymer. In summary, the broad "sugar" wording for the SM moiety in the instant claims does not predictably support hybridization assay practice beyond the more limited form being "furanose".

The enablement rejection is respectfully traversed.

As indicated in the opening remarks of this paper, each of the new independent claims recite "furanosyl moiety," with no reference to or recitation of "sugar moiety."

In light of the language in the new claims, Applicants respectfully request reconsideration and withdrawal of the indefiniteness rejection.

The First Rejection Under 35 U.S.C. §102(b)

Claims 617, 619-633, 635, 637, 639-653, 655, 657, 697, 699-712, 714, 716, 718-731, 733, and 735 stand rejected under 35 U.S.C. §102(b) for anticipation as by Dunn et al. [Cell 12:23 (1977)]. In the Office Action (pages 5-6), the Examiner stated:

Instant claim 617 is directed to an oligo- or polynucleotide which is complementary to a nucleic acid of-interest or a portion thereof which is construed to, indicate that normal base pairing is a characteristic of the claimed oligo- or polynucleotide as is well known for nucleic acid polymers mediated by nucleobase complementarity bonding practice. The oligo- or polynucleotide is also described in instant claim 617 as comprising at least one nucleotide modified via a covalently attached Sig moiety which is covalently attached directly or indirectly (with a chemical linkage) to a PM (phosphate moiety) therein. The Sig moiety is generic except that it must comprise a non-polypeptide, non-radioactive label. Therefore a detectable oligo- or polynucleotide which is covalently attached to a probe oligo- or polynucleotide which is complementary to a nucleic acid of interest is reasonably deemed to anticipate the above claimed subject matter. Dunn et al. at page 24 in Figure 1 depicts an adenovirus RNNSV40 RNA conjugate wherein the SV40 RNA label is non-polypeptide and non-radioactive and attached via a phosphate to the terminal adenovirus RNA oligonucleotide which is complementary to the immobilized Ad2 DNA. This oligo- or polynucleotide thus anticipates the above listed instant claims. It is noted that instant claims such claim 621 etc. limit the chemical linkage option from claim 617 but still are deemed to include the direct phosphate linkage as in the reference and therefore are included as rejected claims herein. Instant

claim 627 is included as rejected over Dunn et al. because the Sig SV40 RNA is chromogenic as being well known to be detectable via UV light.

The first anticipation rejection is respectfully traversed.

As indicated in the opening remarks of this paper, Applicants have defined the non-radioactive label moiety Sig as being "non-polypeptide" and "*non-nucleotidyl*." As discussed in a similar rejection made in related U.S. Patent Application Serial No. 08/486,069, it is believed that the further definition of Sig as being "*non-nucleotidyl*" is a material element altogether lacking in the cited Dunn document.

In view of the presentation of the new claims above, Applicants respectfully request reconsideration and withdrawal of the first anticipation rejection.

The Second Rejection Under 35 U.S.C. §102(a)

Claims 617, 619-633, 635, 637, 639-653, 655, 657, 697, 699-712, 714, 716, 718-731, 733, and 735 stand rejected under 35 U.S.C. §102(a) for anticipation by Hartmann et al. [*Biopolymers* 20:2635-(1981)]. In the Office Action (pages 6-7), the Examiner stated:

The invention is as summarized above. Hartman et al. disclose in the title and abstract the formation of azoRNA for hybridization detection of genes. The Sig label is a yellow chromogenic label which modifies the terminal poly(A) nucleotide as required in the instant claims thus similarly anticipating the instant claims as the above Dunn et al. reference.

The second anticipation rejection is respectfully traversed.

As in the case of the first anticipation rejection, the further definition in the new claims of the non-radioactive label moiety Sig as being "*non-nucleotidyl*" is a

material element altogether lacking in the cited Hartmann document.

In view of the new claims presented above, Applicants respectfully request reconsideration and withdrawal of the second anticipation rejection.

The Third Rejection Under 35 U.S.C. §102(b)

Claims 576,578-593,596,598-613,616,617,619-637, 639-658, 660-674, 677, 679-693,696,697,699-716, and 718-735 stand rejected under 35 U.S.C. §102(b) and (e)(2) for anticipation by Hung et al., U.S. Patent No. 4,224,408. In the Office Action (pages 7-8), the Examiner stated:

Consideration of the above listed instant claims reveals that a non-polypeptide, non-radioactive Sig moiety covalently attached to a terminal nucleotide in an oligo- or polynucleotide or polydeoxyribonucleotide describes a generic DNA polymer. Such a DNA polymer may be envisioned as two segments. The first DNA polymer segment as the oligo- or polynucleotide or polydeoxyribonucleotide is complementary to a specific target nucleic acid via whatever nucleobase sequence is present therein. It is noted that the instant claims are not limited as to any particular sequence of desired target nucleic acid for complementarity in said first DNA polymer segment. The second and remaining DNA segment is covalently attached and thus modifies an terminal nucleotide of the first DNA segment and also is a Sig moiety as being non-polypeptide, non-radioactive and is detectable via its well known UV detectability of its nucleobase content. Thus, the above listed instant claims read on any DNA polymer. Similarly, claims which include either DNA or RNA oligo- or polynucleotides also read on any RNA polymer. The Hung et al. reference discloses both RNA and DNA, as cDNA, polymers in the abstract and throughout its disclosure as part of the

synthesis disclosure therein and therefore anticipates both the instant RNA and DNA type claims as listed above. It is also noted that claims such as instant claims 580-582 etc. do not limit or exclude the basic DNA or RNA polymer embodiments but only limit certain additional options for instant claim embodiments and thus are also rejected hereinunder.

The third anticipation rejection is respectfully traversed.

Similarly, as in the case of the two earlier anticipation rejections, the definition of the non-radioactive label moiety Sig in the new claims as being both "non-polypeptide" and "*non-nucleotidyl*" in nature represents a material element lacking in the cited Hung patent.

In view of the new claims, Applicants respectfully request reconsideration and withdrawal of the third anticipation rejection, thereby placing the new claims, 826-1227, in allowable condition.

An early indication as to the allowable condition of the new claims is respectfully requested.

* * * * *

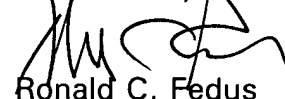
SUMMARY AND CONCLUSIONS

A complete listing of all claims, including new claims 826-1227, is provided above.

The fee for filing new claims 826-1227 is \$1,341, based upon the presentation of 149 additional claims [calculated at \$9 per claim X 149 = \$1,341]. The number of independent claims remains at 12, the number of previously paid for independent claims. The Patent and Trademark Office is hereby authorized to charge the requisite \$1,341 claim fee to Deposit Account No. 05-1135. No other fee or fees are believed due for filing this paper apart from the additional claim fee and extension request (three months). In the event that any other fee or fees are due, however, authorization is hereby given to charge the amount of any such fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,



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CLAIMS TABLE

U.S. Patent Application Serial No. 08/479,997 (Engelhardt et al.)

Filed June 7, 1995

Exhibit 1 To Applicants' April 23, 2004 Amendment Under 37 C.F.R. §1.115 (In Response To The November 26, 2003 Office Action)

Former Claim	New Claim	Changes (Insertion/Deletion/Substitution /Support in Specification
576	826	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl"
578	827	
586	828	insertion of "a saccharide component"
588	829	
589	830	
590	831	
591	832	deletion of "a member selected from the group consisting of" substitution of "or" for "and"
	833	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	834	<i>ibid.</i>
579	835	
580	836	
581	837	
582	838	
583	839	
584	840	substitution of "comprises" for "of Sig includes"
585	841	
585	842	Page 57, Ex. V
592	843	insertion of "the furanosyl moiety of" before "a terminal nucleotide"

593	844	substitution of "furanosyl" for "sugar" and "comprises" for "has"
594	845	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "and 3' "
593	846	substitution of "furanosyl" for "sugar" and "comprises" for "has"
594	847	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "2' and"
	848	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page
	849	Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .); Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates"); and Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")

	850	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluricil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	851	Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-phosphate
	852	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	853	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated"); and</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	854	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
595	855	insertion of "further" before "comprising"

596	856	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "pentose" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl"
598	857	
606	858	insertion of "a saccharide component"
608	859	
609	860	
610	861	
611	862	deletion of "a member selected from the group consisting of" substitution of "or" for "and"
	863	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	864	<i>ibid.</i>
599	865	
600	866	
601	867	
602	868	
603	869	
604	870	substitution of "comprises" for "of Sig includes"
	871	Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .)
585	872	Page 57, Ex. V

	873	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)
612	874	insertion of "the furanosyl moiety"
613	875	insertion of "of said furanosyl moiety;" deletion of "at the 2' position thereof"
614	876	correspondence in both claims with respect to z Insertion of "of said furanosyl moiety;" Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)
	877	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)
	878	<i>ibid.</i>
	879	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page

	880	<p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8;</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .);</p> <p>Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates");</p> <p>Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")</p>
	881	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluracil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	882	<p>Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-Phosphate</p>

	883	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	884	Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated"); Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	885	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
615	886	insertion of "further" before "comprising"
616	887	
617	888	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl"
619	889	
627	890	insertion of deletion of "a component selected from the group consisting of"
629	891	
630	892	
631	893	
632	894	deletion of "a member selected from the group consisting of"
	895	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	896	<i>ibid.</i>
620	897	
621	898	
622	899	
623	900	
624	901	
625	902	substitution of "comprises" for "of Sig includes"
626	903	
626	904	
633	905	insertion of "the furanosyl moiety of"
634	906	substitution of "furanosyl" for "sugar"

635	907	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "and 3' "
	908	Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .)
635	909	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "2' and"
	910	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page
	911	Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .); Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates"); Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")

	912	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluricil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
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	914	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	915	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated");</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	916	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	917	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")

636	918	insertion of "wherein said oligo- or polynucleotide further" substitution of "ribonucleotide" for "deoxyribonucleotide" "ribonucleotide" is recited in claim 617 see also specification, page 53, middle ¶
	919	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid") "ribonucleotide" is recited in claim 617
	920	Page 94, 4th & 5th lines from bottom of page (. . . "when the nucleotide is deoxyribonucleotide" . . .)
637	921	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "pentose" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl"
639	922	
647	923	insertion of "a saccharide component" deletion of "a component selected from the group consisting of"
649	924	
650	925	
651	926	
652	927	deletion of "a member selected from the group consisting of"
	928	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	929	<i>ibid.</i>
640	930	
641	931	
642	932	
643	933	
644	934	

645	935	substitution of "comprises" for "of Sig includes"
	936	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)
646	937	Page 57, Ex. V
	938	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)
653	939	insertion of "the furanosyl moiety"
654	940	insertion of "of said furanosyl moiety;" deletion of "at the 2' position thereof"
655	941	correspondence in both claims with respect to z Insertion of "of said furanosyl moiety;" Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)

	942	<p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8;</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)</p>
	943	<i>ibid.</i>
	944	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page
	945	<p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8;</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .);</p> <p>Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates");</p> <p>Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")</p>

	946	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluricil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
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	948	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	949	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated");</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	950	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
637	951	"ribonucleotide" is recited in claims 637 and 921 see also specification, page 53, middle ¶
	952	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")

	953	"ribonucleotide" is recited in claims 637 and 921 see also specification, page 53, middle ¶
	954	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid") "ribonucleotide" is recited in claims 637 and 921
	955	Page 94, 4th & 5th lines from bottom of page (. . . "when the nucleotide is deoxyribonucleotide" . . .)
658	956	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl" insertion of "a saccharide component"
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669	958	
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	962	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	963	<i>ibid.</i>
661	964	
662	965	
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673	972	insertion of "the furanosyl moiety of"
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675	976	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "2' and"
	977	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page
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	981	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	982	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated");</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	983	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	984	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")

	985	insertion of "wherein said oligo- or polynucleotide further" substitution of "ribonucleotide" for "deoxyribonucleotide" "ribonucleotide" is recited in claim 617 see also specification, page 53, middle ¶
	986	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	987	Page 94, 4th & 5th lines from bottom of page (. . . "when the nucleotide is deoxyribonucleotide" . . .)
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685	1001	substitution of "comprises" for "of Sig includes"

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	1004	<p>Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .)</p>
692	1005	insertion of "the furanosyl moiety"
693	1006	<p>insertion of "of said furanosyl moiety;"</p> <p>deletion of "at the 2' position thereof"</p>
	1007	<p>correspondence in both claims with respect to z</p> <p>Insertion of "of said furanosyl moiety;"</p> <p>Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .)</p>

	1008	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .);
	1009	<i>ibid.</i>
	1010	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page
	1011	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .); Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates"); Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")

	1012	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluricil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	1013	Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-phosphate
	1014	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	1015	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated");</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	1016	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	1017	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	1018	Page 94, 3rd line from bottom of page ("when the nucleotide is a ribonucleotide")

	1019	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	1020	Page 94, 4th & 5th lines from bottom of page ("when the nucleotide is deoxyribonucleotide")
696	1021	
697	1022	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl" insertion of "a saccharide component"
699	1023	
708	1024	
709	1025	
710	1026	
711	1027	deletion of "a member selected from the group consisting of"
	1028	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	1029	<i>ibid.</i>
700	1030	
701	1031	
702	1032	
703	1033	
704	1034	
705	1035	substitution of "comprises" for "of Sig includes"
706	1036	
706	1037	
712	1038	insertion of "the furanosyl moiety of"
713	1039	substitution of "furanosyl" for "sugar"
714	1040	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "and 3' "

	1041	<p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)</p>
714	1042	<p>substitution of "furanosyl" for "sugar" and "comprises" for "has"</p> <p>also deletion of "2' and"</p>
	1043	<p>Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page</p>
	1044	<p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8;</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .);</p> <p>Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates"); and</p> <p>Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")</p>

	1045	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluracil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	1046	Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-phosphate
	1047	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	1048	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated");</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	1049	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	1050	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	1051	Page 94, 3rd line from bottom of page ("when the nucleotide is a ribonucleotide")

	1052	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	1053	Page 94, 4th & 5th lines from bottom of page ("when the nucleotide is deoxyribonucleotide")
716	1054	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl" insertion of "a saccharide component"
718	1055	
727	1056	
728	1057	
729	1058	
730	1059	deletion of "a member selected from the group consisting of"
	1060	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	1061	<i>ibid.</i>
719	1062	
720	1063	
721	1064	substitution of "comprises" for "of Sig includes"
722	1065	
723	1066	
724	1067	
725	1068	recitation for x is found or supported in both claims recitation for y in claim 1068 is supported by one of the members of y in claim 725
725	1069	latter half of claim 725 Page 57, Ex. V
	1070	recitation for "x" is found or supported by claim 1054
731	1071	inserted "the furanosyl moiety of"
732	1072	insertion of "of said furanosyl moiety;" deletion of "at the 2' position thereof"

733	1073	correspondence in both claims with respect to z insertion of "of said furanosyl moiety;" Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)
	1074	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .);
	1075	<i>ibid.</i>
	1076	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page
	1077	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .); Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates"); Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")

	1078	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluricil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	1079	Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-phosphate
	1080	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	1081	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated");</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	1082	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	1083	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	1084	Page 94, 3rd line from bottom of page ("when the nucleotide is a ribonucleotide")

	1085	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	1086	Page 94, 4th & 5th lines from bottom of page ("when the nucleotide is deoxyribonucleotide")
735	1087	
736	1088	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl"
738	1089	deletion of "a component selected from the group consisting of"
742	1090	insertion of "a saccharide component"
744	1091	
745	1092	
746	1093	
747	1094	deletion of "a member selected from the group consisting of"
	1095	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	1096	<i>ibid.</i>
749	1097	Page 57, Ex. V
750	1098	substitution of "comprising" for "is selected from the group consisting of" Page 25, last two lines, through Page 27, 1st ¶
751	1099	Page 57, Ex. V
739	1100	
740	1101	
	1102	Page 11, last ¶ ("that the chemical linkage group be derived from a primary amine, and have the structure -CH ₂ -NH-,")
	1103	Page 11, penultimate ¶ (" -CH = CH-CH ₂ -")
	1104	Page 11, last ¶, through Page 12, 1st ¶

	1105	substitution of "comprises" for "of Sig includes" Original claim 25
741	1106	
741	1107	
751	1108	substitution of "furanosyl" for "phosphate"
752	1109	substitution of "furanosyl" for "sugar"
753	1110	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "and 3' "
	1111	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; Page 12, 2nd full ¶ (The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .);
753	1112	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "2' and"
	1113	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page
	1114	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .); Page 92 ("Ribonucleoside 5'-monophosphates" and "2'- Deoxyribonucleotide 5'-monophosphates"); Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")

	1115	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluricil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	1116	Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-phosphate
	1117	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	1118	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated");</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	1119	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
754	1120	insertion of "further" before "comprising"

755	1121	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl"
757	1122	
761	1123	insetion of "a saccharide component" deletion of "a component selected from the group consisting of"
763	1124	
764	1125	
765	1126	
766	1127	deletion of "a member selected from the group consisting of"
	1128	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	1129	<i>ibid.</i>
768	1130	Page 57, Ex. V
769	1131	substitution of "comprising" for "is selected from the group consisting of" Page 25, last two lines, through Page 27, 1st ¶
770	1132	
758	1133	
759	1134	
	1135	Page 11, last ¶ ("that the chemical linkage group be derived from a primary amine, and have the structure - CH ₂ -NH-,")
	1136	Page 11, penultimate ¶ ("-CH=CH-CH ₂ -")
	1137	Page 11, last ¶, through Page 12, 1st ¶
	1138	Substitution of "comprises" for "of Sig includes" Original claim 25

1121	1139	Found or supported in claim 1121 Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)
	1140	Page 57, Ex. V
	1141	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .); Also found or supported in claim 1121
770	1142	insertion of "the furanosyl moiety of"
771	1143	insertion of "said furanosyl moiety of"
772	1144	insertion of "of said furanosyl moiety" also deletion of "and 3' "
	1145	Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)
772	1146	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "2' and"
	1147	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page

	1148	<p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .);</p> <p>Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates");</p> <p>Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")</p>
	1149	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluracil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	1150	<p>Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-Phosphate</p>

	1151	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	1152	Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated"); Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	1153	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
773	1154	insertion of "further" before "comprising"
774	1155	
775	1156	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl"
777	1157	
781	1158	insertion of "a saccharide component" deletion of "a component selected from the group consisting of"
783	1159	
784	1160	
785	1161	
786	1162	deletion of "a member selected from the group consisting of"
	1163	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	1164	<i>ibid.</i>
778	1165	substitution of "comprises" for "is selected from the group consisting of"
779	1166	
	1167	Page 11, last ¶ (first two lines)
778	1168	Page 11, last ¶ (penultimate line); Page 13, 1st line; and Page 18, 3rd ¶
	1169	Page 11, last ¶, through Page 11, 1st ¶ Page 18, 3rd ¶
	1170	Original claim 25

780	1171	Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .)
780	1172	Page 57, Ex. V
790	1173	insertion of "the furanosyl moiety of"
791	1174	substitution of "furanosyl" for "sugar"
792	1175	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "and 3' "
	1176	Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .)
792	1177	substitution of "furanosyl" for "sugar" and "comprises" for "has" also deletion of "2' and"
	1178	Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page

	1179	<p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .") taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .") taken with structural formula at lower half of page 8; and</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .");</p> <p>Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates");</p> <p>Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")</p>
	1180	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluracil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	1181	<p>Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-phosphate</p>

	1182	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	1183	Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated"); Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	1184	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	1185	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	1186	"ribonucleotide" is recited in claim 785 see also specification, page 53, middle ¶
	1187	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
793	1188	insertion of "further" before "comprising"
788	1189	insertion of "chemical linkage" after "polypeptide"
789	1190	insertion of "chemical linkage" after "polypeptide" substitution of "comprises" for "is selected from the group consisting of"
794	1191	insertion of "or modified nucleotide analog" substitution of "furanosyl" for "sugar" insertion of "a pyrimidine analog," "a purine analog," and "a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization," . . . insertion of "non-nucleotidyl"
796	1192	
800	1193	deletion of "a component selected from the group consisting of" insertion of "a saccharide component"
802	1194	
803	1195	
804	1196	
805	1197	deletion of "a member selected from the group consisting of"

	1198	Page 97, lines 13-14 ("the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide")
	1199	<i>ibid.</i>
797	1200	substitution of "comprises" for "is selected from the group consisting of"
798	1201	
	1202	Page 11, last ¶ (first two lines)
	1203	Page 11, last ¶ (penultimate line); Page 13, 1st line; and Page 18, 3rd ¶
	1204	Page 11, last ¶, through Page 11, 1st ¶ Page 18, 3rd ¶
	1205	Original claim 25
	1206	Found or supported in claim 1191 Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .)
	1207	Page 57, Ex. V
	1208	Found or supported in claim 1191 Pages 2-3 ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at bottom of page 2; Pages 8-9, ("wherein each of x, y, and z represents H – , HO – , " . . .) taken with structural formula at lower half of page 8; and Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H – , HO – , " . . .)
809	1209	insertion of "the furanosyl moiety"
810	1210	insertion of "of said furanosyl moiety"
811	1211	deletion of "both y and" insertion of "of said furanosyl moiety" deletion of "at each of the 3' and 2' positions thereof, respectively"

	1212	<p>Found or supported in claim 1191</p> <p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .)</p>
811	1213	<p>deletion of "both" and "and z"</p> <p>insertion of "of said furanosyl moiety"</p> <p>deletion of "at each of the 3' and 2' positions thereof, respectively"</p>
	1214	<p>Page 97, lines 13-14; Page 99, lines 3-7 from bottom of page</p>
	1215	<p>Pages 2-3 ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at bottom of page 2;</p> <p>Pages 8-9, ("wherein each of x, y, and z represents H- , HO- , " . . .) taken with structural formula at lower half of page 8; and</p> <p>Page 12, 2nd full ¶ ("The letters x, y, and z represent groups attached to the 5', 3', and 2' positions of the sugar moiety. They may be any of H- , HO- , " . . .);</p> <p>Page 92 ("Ribonucleoside 5'-monophosphates" and "2'-Deoxyribonucleotide 5'-monophosphates");</p> <p>Original claims 2 ("said nucleotide is a deoxyribonucleotide") and 3 ("said nucleotide is a ribonucleotide")</p>

	1216	<p>Page 91 ("Two minor purines")</p> <p>Page 31, line 14 ("thymidine analogue")</p> <p>Page 62, Ex. XIII ("5-hydroxymethyluricil" and "5-formyl-2'-deoxyuridine")</p> <p>Page 63, Exs. XIV ("biotinated 5-formyl-2'-deoxyuridine") and XV ("biotin-coupled 5-amino-2'-deoxyuridine");</p> <p>Page 64, Exs. XVI ("5-(oxy)acetic acid-2'-deoxyuridine") and XVII ("biotinyl-1,6-diaminohexane amide coupled to 5-(oxy)acetic acid-2'-deoxyuridine");</p> <p>Page 72, Ex. XXIV ("5-(perfluorobutyl)-2'-deoxyuridine");</p> <p>Page 75, Ex. XXII ("5-(3-amino-1-propenyl)-2'-deoxyuridine-5'-triphosphate");</p> <p>Page 76, Ex. XXXII ("fluorescein coupled to 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP");</p> <p>Page 78, Ex. XXXV ("5-Bromo-2'-deoxyuridine-5'-phosphate");</p> <p>Page 79, Ex. XXXVII ("6-Cyano-2'-deoxyuridine-5'-phosphate"); and</p> <p>Page 80, Ex. XXXVIII ("6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid")</p>
	1217	Page 78, Ex. XXXV ("5-bromo-2'-deoxyuridine-5'-Phosphate
	1218	Page 60, Ex. X ("5-hydroxymethyl-2'-deoxycytidylic acid")
	1219	<p>Page 54, last ¶ ("alkylating agents . . . N2 group of guanine, the N4 group of adenosine or the N4 group of cytosine are alkylated");</p> <p>Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")</p>
	1220	Page 72, Ex. XXV ("toyocamycin" and "tuberacidin")
	1221	Page 94, 3rd line from bottom of page ("when the nucleotide is a ribonucleotide")
	1222	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")

	1223	Page 94, 3rd line from bottom of page ("when the nucleotide is a ribonucleotide")
	1224	Page 95, lines 12-14 (. . . "desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid")
	1225	Page 94, 4th & 5th lines from bottom of page ("when the nucleotide is deoxyribonucleotide")
807	1226	insertion of "chemical linkage" after "polypeptide"
809	1227	substitution of "comprises" for "is selected from the group consisting of"

DNA Replication

DNA Replication

Arthur Kornberg

STANFORD UNIVERSITY



W. H. FREEMAN AND COMPANY
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Cover: A looped rolling circle of the duplex replicative form of phage ϕ X174 (based on an electron micrograph provided by Dr. Jack Griffith). See Figure 11-25 for more detail. (Design by Marjorie Spiegelman.)

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diphosphate kinase in *E. coli*. This ubiquitous enzyme produces nucleoside triphosphates and is thus crucial to all macromolecular syntheses.

12-3 Nucleotide Analogs Incorporated into DNA or RNA^{24,25}

Certain analogs of the nucleoside triphosphates, modified in the sugar or base, are accepted by polymerases for pairing with the DNA template and are incorporated into nucleic acid. They block further chain growth or interfere with nucleic acid functions (Table 12-3; Fig. 12-4).

24. Cozzarelli, N. R. (1977) *ARB* 46, 641.

25. Langen, P. (1975) *Antimetabolites of Nucleic Acid Metabolism*, Gordon and Breach, New York.

TABLE 12-3
Nucleotide analogs incorporated into DNA or RNA

Analog	Incorporated into DNA or RNA	Inhibition
CHAIN TERMINATORS		
2',3'-Dideoxy NTPs	DNA	chain growth, 3'→5' exonuclease
Arabinosyl NTPs (araC, araA)	DNA	chain growth, 3'→5' exonuclease
Cordycepin TP (3'-deoxy ATP)	DNA, RNA	chain growth
3'-Amino ATP	DNA, RNA	chain growth
DEFECTIVE NUCLEIC ACID		
Uracil dNTP (dUTP)	DNA (analog of T)	DNA integrity: excision leads to chain breakage
5-Hydroxyuridine	} TP	RNA, DNA syntheses and functions
5-Aminouridine		
5-Bromouracil	} dNTP	fidelity of replication (mutation), differentiation
5-Iodouracil		
Tubercidin	} "ATP"	RNA, DNA syntheses and functions
Toyocamycin		
Formycin		
7-Deazanebularin	} dNTP	fidelity of replication (mutation)
2-Aminopurine		
2-Aminoadenine (2,6-diaminopurine)		
UNCLASSIFIED		
2'-Deoxy,2'-azidocytidine		initiation of polyoma DNA synthesis; E. coli primase

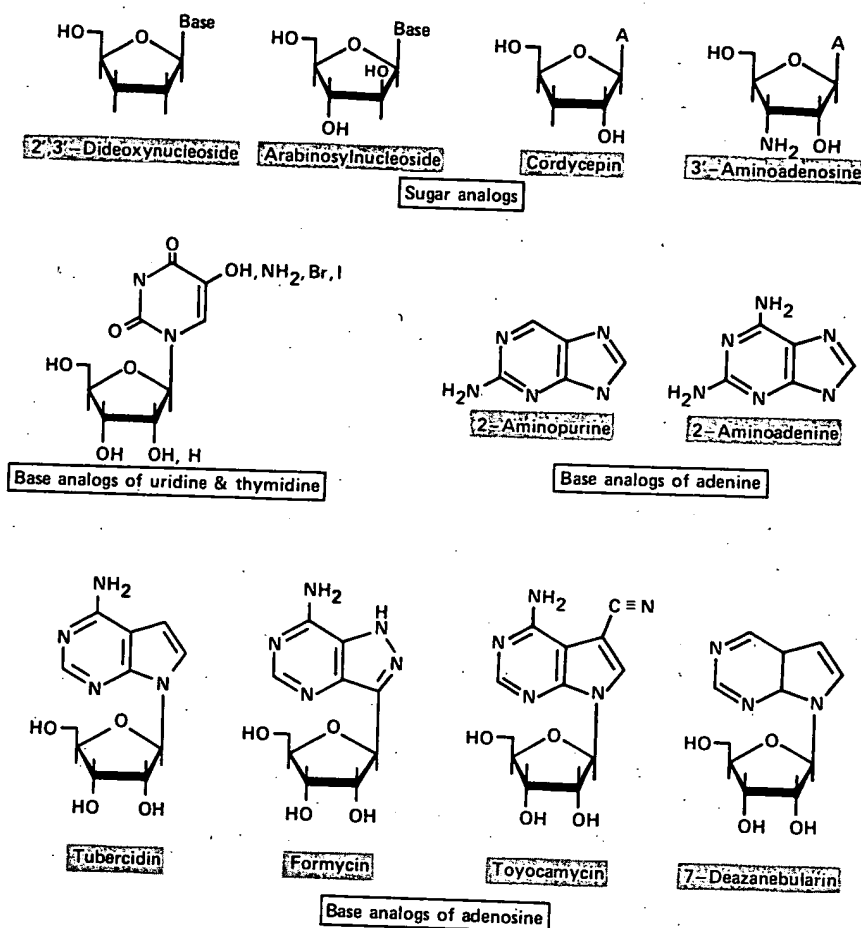


FIGURE 12-4
Nucleotide analogs incorporated into DNA or RNA.

Chain Termination

The 2',3'-dideoxynucleosides, if converted to the triphosphates, are incorporated at a very slow rate. In studies with DNA polymerase I, the discrimination does not appear to be in binding or base pairing of the analog but at a subsequent stage when the inadequacy of the analog as a primer for the next polymerization event is recognized (Ch. 4-7). Because the analog lacks a 3'-hydroxyl group, proofreading excision of the analog is also exceedingly slow, and thus the block of chain growth is maintained. The strikingly specific

inhibition of eukaryotic DNA polymerases β and γ (Ch. 6-1) suggests that strongly competitive binding blocks action of the enzymes.

Arabinosides^{26,27} are notable drugs; the cytosine arabinoside (araC) is used to treat cancer, and the adenine arabinoside (araA)²⁸ is an antiviral agent. Among many possibilities and claims for how the drugs act, the most tenable are based on incorporation of the arabinosides into DNA where they distort the primer-template and block further DNA synthesis by chain termination. The action of araC may be greater in the initiation of DNA chains than in elongation. Nevertheless chain growth is inhibited more markedly with certain prokaryotic (Ch. 5-1, 4) and eukaryotic polymerases (Ch. 6-1) than with others.

Since araC must be in nucleoside triphosphate form to be active, circumstances that favor its conversion by deoxycytidine kinase enhance its clinical value. Competing with this kinase in some cells and tissues is a potent cytidine deaminase, which destroys araC as a drug. Curiously, related arabinosyl analogs, araT and araU, occur in nature in sponges.²⁹

Cordycepin triphosphate³⁰ (3'-deoxy ATP) inhibits chain elongation by RNA and DNA polymerases since its incorporation renders the primer terminus inactive. The nucleoside does not inhibit bacterial growth, probably because it is phosphorylated very poorly. The triphosphate strongly inhibits RNA synthesis in ascites tumor and HeLa cell lines and affects the addition of polyA segments to the 3' end of completed heterogeneous nuclear mRNA. Like 3'-deoxy ATP, 3'-amino ATP³¹ inhibits RNA synthesis in isolated nuclei and extracts of ascites tumor cells by terminal addition to the primer. Although the nucleoside inhibits DNA synthesis in these cells, extracts show no inhibition of DNA synthesis by the triphosphate; ribonucleotide reductase may be the target of in vivo inhibition.

Defective Nucleic Acid

Uracil incorporation into DNA by way of dUTP probably occurs to a significant extent under normal circumstances and can be extensive when the ratio of dUTP to dTTP is elevated (Ch. 2-8; Ch. 11-4). Uracil in DNA is recognized as foreign and excised by N-glycosylases. Mutants defective in the glycosylase can accumulate uracil

26. Matsushita, T., and Kubitschek, H. E. (1975) *Adv. Microb. Physiol.* 12, 247.

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31. Langen, P. (1975) *Antimetabolites of Nucleic Acid Metabolism*, Gordon and Breach, New York.

to levels nearly equimolar with thymine without obvious malfunction of the DNA. Extreme examples are phages in which thymine is completely substituted by uracil or hydroxymethyluracil.

Analogues of uridine and deoxyuridine with 5-hydroxy or 5-amino³¹ substituents inhibit synthesis of DNA, RNA, and protein in *E. coli*, and interfere, in undetermined ways, with the functions of the RNA and DNA molecules into which they are incorporated.

Uridine or deoxyuridine nucleotides containing bromine or iodine³¹ in the 5 position can also cause replication errors when incorporated into DNA; light-induced bromouracil dimers are strongly mutagenic. Phage λ DNA carrying the *lac* operator and containing bromouracil is an order of magnitude more effective in binding *lac* repressor than normal DNA. Thus bromouracil in DNA may also cause altered recognition of specific replication signals.

Tubercidin,³² formycin,³³ toyocamycin,³⁴ and 7-deazanebularin³⁵ are cytotoxic analogs of adenosine. According to in vitro studies with RNA polymerase, they serve, in the form of nucleoside triphosphates, as substitutes for ATP in RNA synthesis. They may inhibit by forming nonfunctional RNA or by acting as antagonists in other ATP reactions. The 2'-deoxy analogs can be formed from the nucleoside triphosphate by ribonucleotide reductase (Ch. 2-6).³⁶ Deazanebularin substitutes for both ATP and GTP in RNA synthesis although it forms only one base pair with uracil and none with cytosine (Ch. 7-5).

2-Aminopurine³⁷ is incorporated into DNA as an analog of adenine. It is mutagenic presumably because of mispairing with cytosine, which can explain its capacity to reverse mutations induced by 5-bromouracil. As a powerful inhibitor of adenosine deaminase, its immunosuppressive effects may be anticipated (Ch. 2-6).

2-Aminoadenine (2,6-diaminopurine) as the deoxynucleoside triphosphate is an analog of dATP, and effectively substitutes for it with DNA polymerase I.³⁸ In substituting, it forms three hydrogen bonds with thymine and may give rise to mispairing with cytosine. Remarkably, 2-aminoadenine has been found in place of adenine in the duplex DNA of a phage that infects blue-green algae.³⁹ Ele-

31. Langen, P. (1975) *Antimetabolites of Nucleic Acid Metabolism*, Gordon and Breach, New York.

32. Nishimura, S., Harada, F., and Ikehara, M. (1966) *BBA* 129, 301.

33. Ikehara, M., Murao, K., Harada, F., and Nishimura, S. (1968) *BBA* 155, 82; Ward, D. C., Cerami, A., Reich, E., Acs, G., and Altwerger, L. (1969) *JBC* 244, 3243.

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39. Kirnos, M. D., Khudyakov, I. Y., Alexandrushkina, N. I., and Vanyushin, B. F. (1977) *Nat.* 270, 369.

vated DNA-melting temperature indicates triply hydrogen-bonded base pairings with thymine. In this unique example of the complete substitution of a DNA purine with a novel base, it will be interesting to learn how dATP is excluded from DNA and what influence 2-aminoadenine has on the genetic stability of these phages.

Unclassified

*2'-Deoxy-2'-azidocytidine*⁴⁰ appears to be an analog of either ribo- or deoxyribonucleosides. It inhibits primase (Ch. 11-10)⁴¹ and an early step in the synthesis of viral polyoma DNA in hamster ovary cells, possibly at the initiation of each replication cycle. Although nucleoside diphosphates bearing a 2'-azido group specifically inactivate ribonucleotide reductase in vitro (by destroying the free radical in the enzyme), the in vivo effect cannot be attributed to this action. It is not yet known whether the analog is incorporated into DNA or RNA.

12-4 Inhibitors That Bind to or Modify DNA⁴²

Although DNA is a chemically unreactive molecule, the need to preserve its conformation and continuity for extraordinary lengths makes it vulnerable to agents that bind to it noncovalently or introduce occasional covalent modifications. Clustering of AT and GC pairs in regions that serve as origins of replication, promoters of transcription, and other vital signals may offer especially sensitive targets for certain of these agents (Table 12-4; Fig. 12-5).

Noncovalent DNA Binders

*Actinomycin D*⁴³ is one of the most extensively used and studied inhibitors of nucleic acid synthesis, particularly RNA synthesis. It has a lesser effect on DNA synthesis. An attractive model, based on x-ray diffraction patterns of nucleotide-drug crystals, pictures intercalation of the planar phenoxazone ring system between alternating base pairs of poly dGC and hydrogen-bond linkage of the cyclic peptide portion with the 2-amino group of guanine in the

40. Skoog, L., Bjursell, G., Thelander, L., Hägerström, T., Hobbs, J., and Eckstein, F. (1977) *EJB* 72, 371; Bjursell, G., Skoog, L., Thelander, L., and Söderman, G. (1977) *PNAS* 74, 5310.

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Chapter 1

Why Study Nucleotide and Nucleic Acid Structure?

Before embarking on a description of nucleotide and nucleic acid structures, let us examine the biological importance of this class of molecules and find out why their structural principles should be known at the atomic level.

Nucleotides have many functions in living organisms. The hereditary material, deoxyribonucleic acid (DNA), is a linear polymer built up of monomeric units, the nucleotides. Even if these units were not constituents of DNA, they would nevertheless be among the most important molecules in biology.

A nucleotide consists of three molecular fragments: sugar, heterocycle, and phosphate. The sugar, ribose or deoxyribose, is in a cyclic, furanose form and is connected by a β -glycosyl linkage with one of four heterocyclic bases to produce the four normal nucleosides: adenosine, guanosine, cytidine, and thymidine (uridine in ribonucleic acid, RNA). If the 3'- or 5'-hydroxyl group of sugar is phosphorylated, we have a nucleotide. This unit, the nucleotide, is not only the building block of the polynucleotides DNA and RNA but it also exhibits independent functions.

For example, with adenosine derivatives displayed in Figure 1-1, we can show that, depending on chemical modifications, adenosine adapts to several, dramatically different biochemical roles in life. As di- and triphosphates, adenosine acts as an energy pool for many enzymatic processes and for muscle work. The importance of adenosine triphosphate is demonstrated by its turnover rate in humans: about one body weight per day per person. The 3', 5'-cyclic phosphate of adenosine is the "second hormonal messenger," controlling and mediating the activities of peptide hormones. In the form of puromycin, adenosine is a potent inhibitor of protein biosynthesis and as arabino- or 8-azaderivatives, adenosines display antibiotic activities. Adenosine diphosphate, equipped at the terminal phosphate with certain biological molecules, is a constituent of both coenzymes A and NAD⁺ and is essential for the proper functioning of enzymes which require these cofactors.

To understand the biological function of a nucleotide, we must know its structural features. With the adenosine derivatives mentioned above, we know the *chemical structure* and we know the function. But why and how do they function? Why, for instance, is 8-azaadenosine an antibiotic even though, from a chemical point of view, the isoelectronic substitution of

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position but differ from each other in the type of functional group that they contain.

function of state See state function.

fungi Of, or pertaining to, fungi.

fungicide An agent that kills fungi.

fungus (*pl* fungi) A plant protist that is non-photosynthetic and that is devoid of chlorophyll; fungi generally contain a mycelium and are frequently coenocytic.

furan A heterocyclic compound, the structure of which resembles the ring structure of the furanoses.

furanose A monosaccharide having a five-membered ring structure.

furanoside A glycoside of a furanose.

fused gene A hybrid gene produced by linking a gene of interest (for example, a mammalian gene) to some other gene (for example, a plasmid gene) using methods of recombinant DNA technology. *Aka* hybrid gene. See also fusion gene.

fused protein A hybrid protein molecule, consisting of two linked and different proteins, and produced from a fused gene. *Aka* hybrid protein.

fused ring A ring that has two or more atoms in common with another ring.

fused rocket immunoelectrophoresis See rocket electrophoresis.

fusel oil A group of compounds formed as side products during alcoholic fermentation; the mixture consists mainly of amyl, isoamyl, isobutyl, and propyl alcohols.

fusidic acid A steroid antibiotic, produced by *Fusidium coccineum*, that inhibits protein

synthesis in both prokaryotes and eukaryotes by interfering with elongation factor G (translocase).

fusiform Spindle-shaped; tapered at each end.

fusion See plasmid fusion; replicon fusion; gene fusion; nuclear fusion.

fusion gene A hybrid gene consisting of parts of two others genes. A fusion gene can be formed by deletion of a chromosomal segment between two genes or by crossing over. See also fused gene.

fusogenic agent An agent, such as polyethylene glycol or Sendai virus, that induces cell fusion.

futile cycle A substrate cycle in which the two opposing reactions occur at comparable rates in the same cell. Such a cycle accomplishes nothing except the waste of the free energy difference between the two reactions or, possibly, the generation of some heat. For example, the reaction $\text{glucose} + \text{ATP} \rightleftharpoons \text{glucose-6-phosphate} + \text{ADP}$ together with the reaction $\text{glucose-6-phosphate} + \text{H}_2\text{O} \rightleftharpoons \text{glucose} + \text{P}_i$ leads only to the net reaction of $\text{ATP} \rightleftharpoons \text{ADP} + \text{P}_i$.

fuzzy coat CELL COAT.

F value 1. A ratio of two variances; See *F* test.

2. The time required, when treating an aqueous suspension at 121°C, to heat inactivative (kill) the entire population of viable bacterial cells or spores.

Fv fragment The N-terminal portion of the Fab fragment of the immunoglobulins; it consists of the variable portions of one heavy and one light chain.